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Variants of the *MTHFR* gene and susceptibility to acute lymphoblastic leukemia in children: A synthesis of genetic association studies

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is a complex disease with genetic background. The genetic association studies (GAS) that investigated the association between ALL and the *MTHFR C677T* and *A1298C* gene variants have produced contradictory or inconclusive results. *Materials and methods*: In order to decrease the uncertainty of estimated genetic risk effects, a meticulous meta-analysis of published GAS related the variants in the MTFHR gene with susceptibility to ALL was conducted. The risk effects were estimated based on the odds ratio (OR) of the allele contrast and the generalized odds ratio (OR_G). Cumulative and recursive cumulative meta-analyses were also performed.
Results: The analysis showed marginal significant association for the *C677T* variant, overall [OR = 0.91 (0.82–1.00) and OR_G = 0.89 (0.79–1.01)], and in Whites [OR = 0.88 (0.77–0.99) and OR_G = 0.85 (0.73–0.99)]. The *A1298C* variant produced non-significant results. For both variants, the cumulative meta-analysis did not show a trend of association as evidence accumulates and the recursive cumulative meta-analysis indicated lack of sufficient evidence for denying or claiming an association. *Conclusion*: The current evidence is not sufficient to draw definite conclusions regarding the association of *MTHFR* variants and development of ALL.

1. Introduction

Acute lymphoblastic leukemia (ALL), is a form of leukemia characterized by excess lymphoblasts. ALL is most common in childhood with a peak incidence at two to five years of age and the cure rate is about 80% [1]. In ALL, malignant immature white blood cells excessively multiply in the bone marrow and cause damage and death by crowding out normal cells in the bone marrow, and by infiltrating to other organs [2]. The exact biologic mechanisms and etiology of ALL is not known. However, the wide spectrum of molecular diversity of ALL indicates that its pathogenesis involves an interaction between inherited predispositions, exposure to exogenous factors (e.g. radiation and chemicals) with leukemogenic potential and impaired hematopoietic development [3,4].

Methylenetetrahydrofolate reductase (*MTHFR*) is a critical enzyme in one-carbon metabolism. *MTHFR* catalyzes the irreversible conversion of 5,10-methylenetrahydrofolate to 5-methyltetrahydrofolate, which is the predominant circulating form of folate and serves as the carbon donor for the remethylation of homocysteine to methionine [5]. The *MTHFR* [6,7] gene is localized on chromosome 1p36.3. Two common polymorphisms (variants) have been described in the *MTHFR* gene which are single nucleotide substitution resulting in amino acid changes: (i) the *MTHFR C677T* (exon 4 at codon 222), which is a C > T substitution at position 677 resulting in an alanine to valine substitution, and (ii) the *A1298C* (exon 7 at codon 429), causing a glutamate to alanine (A > C) substitution. Both of these polymorphisms have an impairment in the enzyme activity. The 677T allele and 1298C allele have been found to result in decreased enzyme activity [6,7] leading to increased homocysteine levels and thus to an imbalance in plasma folate concentration. *A1298C* influences the activity of the enzyme to a lesser extent than the *C677T* polymorphism does

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[5]. These two polymorphisms are implicated with the development of ALL and have already been associated with different disorders/diseases [8–11]. Although they have been associated with increased risk for some cancers (e.g. breast and gastric), they have been linked to a decreased risk of adult and pediatric ALL [8]. This protective effect might be due to a more efficient DNA synthesis and repair since the excessive 5,10-methylenetrahydrofolate levels (which cannot be converted through the less active MTHFR enzyme) are used for the conversion of uracil to thymine and for purine synthesis [12–14]. However, other functional polymorphisms in genes associated with impaired folate metabolism, may also contribute to the risk of cancer and other disorders [15,16].

The genetic association studies (GAS) that investigated the association between ALL and the *MTHFR C677T* and *A1298C* variants have produced contradicted or inconclusive results, partly because the studies had limited sample sizes and their power was not adequate to demonstrate significant association. In addition, the studies involved different populations and sampling strategies making the interpretation of results difficult. Two meta-analyses [8,17] regarding the role of *MTHFR C677T* and *A1298C* variants have been previously carried out, but included the relative scarce information available at that time.

In the present study, a meticulous meta-analysis [18], including cumulative and recursive cumulative meta-analysis [19,20], was performed for GAS related the *MTHFR C677T* and *A1298C* variants with ALL. Aim of the meta-analysis was to provide an estimated pooled genetic risk effect with decreased uncertainty and to explore whether there is sufficient evidence to deny or claim association. The consistency of genetic effects across traditionally defined ethnicities was also examined.

2. Materials and methods

2.1. Identification and eligibility of relevant studies

GAS that investigated the association of the *MTHFR C677T* or *A1298C* genetic variants with the risk of developing ALL published in English before March 2011 were considered in the meta-analysis. The studies were identified by systematically searching the PubMed database and the HuGE Navigator. The following search criterion was used: (“methylenetetrahydrofolate reductase” or “*MTHFR*” or “*C677T*” or “*A1298C*”) and (“leukemia” or “acute lymphocytic leukemia” or “acute lymphoblastic leukemia”) and (“gene” or “polymorphism” or “genetic variant”) and (“association” or “risk” or “susceptibility”). Then, the abstracts were retrieved and screened to assess their appropriateness for inclusion in the meta-analysis. After the abstract screening, the articles were read in their entirety in order to assess their eligibility for the meta-analysis. Finally, all the references in the eligible articles were extensively reviewed to identify additional published articles not indexed by PubMed database or HuGE Navigator.

GAS provided the genotype distribution of *C677T* and *A1298C* variants in children with ALL (including all ALL subtypes) and in control subjects free of any malignancies were eligible for inclusion in the meta-analysis. In studies with overlapping cases or controls, the largest in size study was included in the meta-analysis. Family-based studies were not considered because of different design considerations.

2.2. Data extraction

From each study the following information was extracted: first author, journal, year of publication, ethnicity of study population, demographics, ALL subtype and genotype distribution.

2.3. Data synthesis and analysis

The meta-analysis examined the association between each variant and the risk of ALL based on the allele contrast (mutant type vs. wild type) [18]. Then, the associations were expressed as pooled odds ratios (OR) with the corresponding 95% confidence intervals (CI) [18]. In addition, the generalized odds ratio (OR_G) was calculated for each study [21]. The OR_G provides an estimate of the overall risk effect by utilizing the complete genotype distribution. The OR_G express the probability of a subject being with ALL relative to probability of being free of disease, given that the subject with ALL has a higher mutational load than the non-diseased [21].

In synthesizing the studies, the random effects (RE) pooled OR of the allele contrast and the RE OR_G were used [18,21,22] since RE is more conservative than the fixed effects OR. The RE model incorporates in the estimates the between study variability [18]. The heterogeneity between studies was tested using the Q-statistic [23] and the heterogeneity was considered significant at $P_Q < 0.10$. The heterogeneity was also quantified using the I^2 metric which takes values between 0% and 100% with higher values denoting greater degree of heterogeneity [24].

In order to evaluate the trend of association, a cumulative and recursive cumulative meta-analysis was conducted based on the RE OR for the allele contrast [18,20]. In cumulative meta-analysis, studies were chronologically ordered by publication year and the OR is obtained at the end of each year (each information step) [18]. In recursive cumulative meta-analysis, the relative change in OR at each information step was calculated. Cumulative and recursive cumulative meta-analyses, provide the framework for updating the estimated genetic risk effect in time and they measure the changes in risk effect as evidence accumulates [18]. In particular, the cumulative meta-analysis indicates the trend in the risk effect and recursive cumulative meta-analysis indicates the stability in the risk effect. The differential magnitude of effect in large versus small studies for the allele contrast was checked using the Harbord’s test [25].

The meta-analysis consisted of the overall analysis, which includes all available data and subgroup analysis by ethnicity (“racial” descent). A sensitivity analysis which examines the effect of excluding specific studies was also considered [18]. The distribution of the genotypes in the control group was tested for conforming to the Hardy–Weinberg equilibrium (HWE) rule using an exact test [18]. Deviation from HWE indicates possible genotyping errors and/or population stratification and studies with controls deviated from HWE were subjected to a sensitivity analysis [18]. Analyses were performed using Meta-Analyst V.3 (Evidence-Based Practice Centers, Tufts Medical School) and CUMAGAS (http://biomath.med.uth.gr). The OR_G (individual and pooled) was estimated using ORGGASM (http://biomath.med.uth.gr) [21].

3. Results

3.1. Eligible studies and studies’ characteristics

The literature review identified 98 articles in PubMed that met the search criteria. The articles identified in HuGE Navigator were already traced in PubMed. Data from 23 articles met the inclusion criteria. In two studies [26,27] the subjects were overlapped, and therefore, the largest study was included in the meta-analysis [27]. Fig. 1 presents a flow chart of the retrieved and excluded studies with specification of reasons.

The characteristics of the individual studies included in the meta-analysis are provided in Table 1. All 23 studies dealt with the variant *C677T* and 19 with the variant *A1298C*. The studies provided 4517/7117 cases/controls for *C677T* and 4360/6717